REMARKS

Claims 1-6 are pending in this application. Claim 1 has been amended to recite the subject matter of claim 2, which has been canceled in favor of amended claim 1. Claim 5 has been amended to change the dependency to claim 1. After entry of this Amendment, claims 1 and 3-6 will be pending for consideration. Applicants urge the Examiner to enter this amendment, which places this case in condition for allowance.

I. Claim Rejections - 35 U.S.C. § 112

Claims 2-3 and 5-6 stand rejected under 35 U.S.C. § 112, first paragraph, for the reasons stated in the prior Office Action. The Examiner asserts that the invention appears to employ specific mutant strains to obtain certain amounts of canthaxanthin, and that it is not clear if the written description is sufficiently repeatable to avoid the need for a deposit.

The Examiner asserts that Applicants argue on one hand that the Examples demonstrate that the mutated microorganisms can be easily derived (Response, page 4), yet, on the other hand, argue that the Tsubokura *et al.* method of mutating does not produce the required productivity, even though substantially the same method of mutagenesis is used.

Further, the Examiner asserts that it cannot be readily assessed how one of ordinary skill in the art can obtain the mutants required to make the process of claims 2-3 and 5-6 without undue experimentation. According to the Examiner, the mutagenesis protocol followed relies on chemical mutagenesis with nitrosoguanidine, the effects of which are random and unpredictable. The Examiner therefore concludes that, in the absence of a specific screening method, it is deemed that the specific strains actually produced are not readily available to the public and should be deposited for this purpose.

Applicants respectfully but vigorously traverse this rejection and offer the following explanation that should resolve any perceived inconsistency.

Any skilled person in the art can obtain expected mutants with high reproducibility by selecting a mutant that produces canthaxanthin, the mass percentage of which relative to the total amount of carotenoid produced, is at least 40% by mass. The probability of obtaining an expected mutant is 1/400 in Example 1 of the present application, 2/1000 in Example 2, and 1/300 in Example 3, which is very high and highly reproducible.

A skilled person in the art routinely evaluates and screens a several hundreds to several thousands of mutants to obtain a desired mutant. Thus, the amount of experimentation needed to support the full scope of the claimed invention is not undue in view of the Examples.

In support of the above assertions with regard to what would have been "routine" testing to the skilled artisan, applicants submit herewith a copy of page 26 of Crueger, W., *et al. Biotechnology: a textbook of industrial microbiology*, 2nd Ed., Editor of English Edition: Thomas D. Brock Sinauer Associates, Inc. Sunderland, MA 01275 (1990). Here, the authors discuss routine methods for screening mutant microbiological strains. They state that "[n]ormally, several hundred to several thousand isolates per mutation cycle must be tested." In view of this passage, it is clear that the amount of testing required to obtain the mutants of the present invention is well within the normal range for this art.

In contrast, Tsubokura *et al.* do not teach or suggest such a screening method to select a mutant that produces canthaxanthin, the mass percentage of which relative to the total amount of carotenoid produced is at least 40% by mass. This is the reason why a skilled person cannot obtain a mutant, according to Tsubokura *et al*, that produces canthaxanthin the mass percentage of which relative to the total amount of carotenoid produced, is at least 40% by mass.

In view of these comments and the above amendment, applicants respectfully request the Examiner to reconsider and withdraw the rejections for lack of written description.

III. Claim Rejections - 35 U.S.C. § 102

Claims 1 and 4 stand rejected under 35 U.S.C. 102 (b) as being anticipated by Tsubokura *et al.* The Examiner asserts that the claims are directed to a process of mutating and culturing a strain such as a mutant of strain E-396 for the production of canthaxanthin. The Examiner further asserts that Tsubokura *et al.* teach a process of mutating and culturing a strain such as E- 396, wherein the mutant strain Y-1071 is cultured for the production of canthaxanthin as claimed. *See, e.g.*, Example 3.

According to the Examiner, regarding the higher yields of canthaxanthin, a comparison between each of the ratios obtained in Table 4 and each of the ratios obtained in Table 7 shows "a higher mass percentage of canthaxanthin relative to that produced by the parent strain".

Applicants respond by noting that the Examiner has not rejected claim 2 for lack of novelty. Applicants further respond by directing the Examiner's attention to the amendment to claim 1, which now recites the subject matter of claim 2. Thus, Applicants believe that the Examiner's rejection is now moot.

However, Applicants do wish to address the Examiner's allegation that Tables 4 and 7 of Tsubokura *et al.* show "a higher mass percentage of canthaxanthin relative to that produced by the parent strain" without showing calculated value of mass percentage of canthaxanthin relative to the total amount of carotenoid produced. The calculated values are shown in the following TABLE 1 and TABLE 2. TABLE 1 corresponds to Table 4 of Tsubokura et al. TABLE 2 corresponds to Table 7 of Tsubokura et al. Comparing TABLE 1 and TABLE 2, the calculated value of mass percentage of canthaxanthin relative to the total amount of carotenoid produced, i.e. 100 X [D]/[T], obtained by mutant Y-1071 is higher than the percentage obtained by

the parent strain E-396 in 4 cases out of 6, namely in the cases where the concentration of dissolved oxygen is 15, 25, 30, or 35%. However, the difference is very small. Besides, even the highest value of 100 X [D]/[T] obtained by mutant Y-1071 is as low as 14.5%. According to the present invention, the values of 100 X [D]/[T] obtained by mutants are more than 60% as are shown in Tables 2-5 of the specification. Hence, it is apparent that the mutants selected according to the present invention are completely different ones from mutant Y-1071 of Tsubokura *et al.*

TABLE 1 Canthaxanthin Obtained By Using E-396

Concentration of Dissolved Oxygen (%)	Canthaxanthin (mg/L) [D]	Total Carotenoid (mg/L) [T]	100 X [D]/[T] (%)
5	3.3	17.8	18.5
15	3.7	27.2	13.6
20	2.0	27.2	7.4
25	1.7	32.1	5.3
30	0.8	21.3	3.8
35	0.7	21.1	3.3

TABLE 2 Canthaxanthin Obtained By Using Y-1071

Concentration of Dissolved Oxygen (%)	Canthaxanthin (mg/L) [D]	Total Carotenoid (mg/L) [T]	100 X [D]/[T] (%)
5	29.8	218.4	13.6
15	38.5	265.7	14.5
20	17.2	272.0	6.3
25	17.8	280.1	6.4
30	9.3	217.3	4.3
35	8.4	219.8	3.8

Applicants submit further evidence to show that the mutant of the present invention is quite different from Y-1071 of Tsubokura *et al.* by comparing the composition of carotenoid produced by using the parent strain E-396 (Table 4 of Tsubokura *et al*), the mutant Y-1071 (Table 7 of Tsubokura at al), and the mutant of Example 1 of the present application at the concentration of dissolved oxygen of 5% which gives the highest value of 100 X [D]/[T] for the parent strain E-396 (see TABLE 1).

Values of 100 X [X]/[T], wherein X is the amount of each carotenoid compounds and T is the total amount of carotenoid compounds obtained by using E-396 or Y-1071 at the concentration of dissolved oxygen of 5%, are calculated from the data shown in Tables 4 and 7 of Tsubokura *et al.* The outcomes are shown in the following TABLE 3 together with the corresponding data for the mutant of Example 1 of the present application shown in Table 2.

TABLE 3 Ratio of Each Carotenoid Compounds

	100 X [X]/[T] (%)		
	E-396	Y-1071	Example 1 of Present Invention
β-Carotene	28.1	36.2	7.1
Echinenone	9.0	8.2	11.9
3-	2.8	1.5	0.0
Canthaxanthin	18.5	13.6	64.3
Adonirubin	22.5	21.2	14.3
β-Cryptoxanthin	0	0	0.0
Astaxanthin	15.7	16.2	2.4
Asteroidenone	0.6	0.3	0.0
Adonixanthin	2.2	2.4	0.0
Zeaxanthin	0.6	0.4	0.0

TABLE 3 shows that almost no changes are observed in the value of 100 X [X]/[T] for canthaxanthin and the carotenoid composition between E-396 and Y-1071. On the contrary, in case of the mutant of Example 1 of the present invention, the value of 100 X [X]/[T] for canthaxanthin drastically improves, and it can be seen also that the carotenoid composition obtained by the mutant of Example 1 of the present invention is entirely different from that of E-396. Namely, the mutant such as Y-1071 and the canthaxanthin producing mutant of present invention are completely different in their characteristics. Based on this evidence, a skilled person would never expect to obtain from Y-1071 the mutant of the present invention, which drastically improves the ratio of canthaxanthin to the carotenoid content.

III. Claim Rejections - 35 U.S.C. § 103

Claims 1-6 stand rejected under 35 U.S.C. 103 (a) as being unpatentable over Tsubokura *et al.* The Examiner asserts that the claims are directed to a process of mutating and culturing a strain such as a mutant of strain E-396 for the production of canthaxanthin in certain amounts. The Examiner further asserts that Tsubokura *et al.* teach a process of mutating and culturing a strain such as E- 396, wherein the mutant strain Y-1071 is cultured for the production of canthaxanthin as claimed. See, *e.g.*, Example 3.

According to the Examiner, the reference differs from the invention as claimed in that the amounts of canthaxanthin and other pigment products are not the same as claimed. The Examiner further contends, however, that the reference teaches methods of mutation suitable to obtain further mutants and in addition discloses that the manipulation of process conditions, such as concentration of dissolved oxygen affects the results obtained.

The Examiner also states that one of ordinary skill in the art would have had a reasonable expectation of success in using the process of Tsubokura *et al.* of mutation and selection and of manipulating oxygen content in the medium to obtain greater yields of canthaxanthin with the mutant obtained, for example.

The Examiner concludes that it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to modify the process of Tsubokura *et al.* by subjecting the strains known to produce canthaxanthin to further mutation and selection, if necessary, and/or manipulating process conditions to maximize the production of the valuable carotenoid canthaxanthin. Applicants strongly traverse this rejection, particularly in view of the amendment to claim 1 and the comparative data discussed above. The fact that tools existed for carrying out the invention does not render the invention as presently claimed obvious. The Examiner's case is based upon hindsight of knowing the invention, which is an impermissible basis for obviousness.

Applicants also wish to address the Examiner's responses to their prior arguments. Specifically, the Examiner has stated that Applicants have not substantiated that all microorganisms having the required homology would produce the higher mass of canthaxanthin relative to that produce by the parent strain. Applicants disagree. The Examples of the present application describe two strains: E-396 and A-581-1. The fact that two strains, which have been isolated quite separately from soil, can be equally applied to the present invention, strongly suggests that the method of the present invention can be applied to all of the same kind of astaxanthin-producing strains which form a group, as is explained in pages 3-6 of the specification. E-396 strain is classified scientifically by a minute analysis to *Paracoccus carotuvfaciens* (International Journal of Systematic Bacteriology (1999), 49, 277-282). Other strains which are classified in *Paracoccus* are listed in TABLE 4 (below). The homology of their nucleotide sequence of 16S ribosomal RNA with that of E-396 strain, and the existence of the production report of astaxanthin are also shown in TABLE 4. There exists various kinds of strains in *Paracoccus.* Among them, strains which are known as producing astaxanthin are limited to strains which are very highly homologous to E-396 and A-681-1. So these strains can be treated as a one group. Among these highly similar strains, their pathway for camtenoid biosynthesis resemble to each other as a matter of course. Hence, it is evident that a mutant giving high canthaxanthin ratio can be

obtained by a similar method. Accordingly, the full breadth of the limitation of "homology of 98% or higher" is well-supported by the specification.

TABLE 4

	Homology with E-396 (%)	Production Report of Astaxanthin
Paracoccus carotinifaciens E-396	100.0	Yes
Paracoccus marcusii DSM11574	99.7	Yes
Paracoccus haeundaensis BC 74171	99.6	Yes
Paracoccus sp. A-581-1	99.4	Yes
Paracoccus seriniphilus MBT-A4	97.0	No
Paracoccus alcaliphilus JCM 7364	95	No
Paracoccus aminovorans JCM 7685	95	No
Paracoccus aminophilus JCM 7686	95	No
Paracoccus zeaxanthiaifaciens ATCC21588	95	No
Paracoccus solventivorans DSM 6637	95	No
Paracoccus kocurii JCM 7684	94	No
Paracoccus akrenifer DSM 11593	94	No
Paracoccus denitrificans ATCC17741	94	No
Paracoccus thiocyanatus IAM 12816	94	No
Paracoccus yeei ATCC BAA-599	94	No
Paracoccus versutus ATCC 25364	93	No
Paracoccus pantotrophus ATCC35512	93	No
Paracoccus kondratievae VKMB-2222	93	No
Paracoccus methylutens VKMB-2164	92	No

The Examiner has further alleged the following with regard to Applicants' prior response:

> "It is noted that applicant has not demonstrated that the asserted productivityis the effect of the mutation rather than of process conditions that achieve thehigher results of canthaxanthin for any and all mutants obtainable."

In response, applicants direct the Examiner to Japanese Patent Publication (Unexamined Application) No. 2005-087097, which discloses at Table 3, the outcomes of culturing E-396 under the same conditions of the present application at Example 1. (The English language counterpart to this reference, US Patent Application No. 2007/0105189 is attached.) The outcomes of the above JP Unexamined Application Publication No. 2005-087097 is shown in the following TABLE 5. When these data are compared with the data of Table 2 of the present application, it is apparent that the rise of ratio of canthaxanthin is attributable not to process conditions but to mutation.

TABLE 5

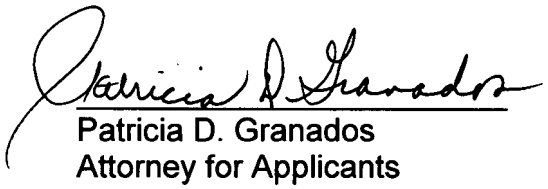
Carotenoid compound	Concentration of product per culture solution (mg/L)	Ratio of product % by mass
β-Carotene	1.6	6.6
Echinenone	1.8	7.4
3-Hydroxyechinenone	0.4	1.6
Canthaxanthin	1.6	6.6
Adonirubin	1.0	4.1
β-Cryptoxanthin	-	
Astaxanthin	6.4	26.3
Asteroidenone	115	6.2
Adonixanthin	8.6	35.4
Zeaxanthin	1.4	5.8

In view of the above amendment, explanations and comparative data, applicants respectfully request the Examiner to reconsider and withdraw the rejection under 35 USC § 103.

CONCLUSION

In light of the above amendments and comments, applicants respectfully request that all rejections and objections be withdrawn and that a timely Notice of Allowance should be issued in this application. Should the Examiner have any questions, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,



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